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Prevalence of *Campylobacter* Spp. in Marketable Milk and Some Milk Products in New Valley Governorate, Egypt

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Abstract

The current study aimed to determine the prevalence of *Campylobacter* in milk and milk products as well as the determination of isolated *Campylobacter* in Egypt's New Valley Governorate. 150 random samples of marketable milk (dairy farm and dairy shop) and some milk products: soft cheeses (Kareish, Domiati) and Ice cream (30 for each) were obtained from various locations in the New Valley Governorate. *Campylobacter* spp. were found in 6.6% of marketable milk from dairy farms. In addition, *Campylobacter* spp. were found in 3.3% of soft cheese samples (Kareish cheese). while they couldn't be detected in Domiati cheese samples. Moreover, *Campylobacter* spp. were found in 10% of ice cream samples. *Campylobacter* organisms were identified as *C. coli* (2%) and *C. jejuni* (2%) in marketable milk (dairy farm and dairy shop) and some milk products. In conclusion, *Campylobacter* species is detected in some milk and some milk products in New Valley governorate. So, restricted milk hygiene must be applied during milking, manufacturing, and marketing milk and its products.

KEYWORDS
Marketable milk, *Campylobacter* spp., *C. coli*., *C. jejuni*

INTRODUCTION

Campylobacter is a Greek name consisting of campy; which refers to curves, and bacter; which refers to a rod, and characterizes the appearance of the organisms. *Campylobacter* bacteria are all tiny, non-spore-forming Gram-negative bacteria with a distinctive curved, S-shaped, or spiral morphology (1.5–6.0 m long and 0.2–0.5 m wide). They are mobile and microaerophilic organisms that require a 5% O2, 10% CO2, and 85% N2 environment (Ebraheem, 2011).

In humans, the massive majority of *Campylobacter*iosis cases are gastrointestinal infections and, in most cases, the incubation period lasts 2 to 5 days. but may differ from one to eleven days. Sudden onset of diarrhea, which may be watery or bloody, is the most common symptom. Other symptoms are abdominal cramps, fever, headache, nausea, myalgia and vomiting. The illness is most often self-limiting, with symptoms declining after a few days up to two weeks (Streur, 2011).

Campylobacter concentrations in raw milk are normally low, yet sufficient to induce disease, given the low infective dosage for *Campylobacter*iosis. Because of its widespread presence in nature and in food animals, and being factors affecting human and animal health, especially *C. jejuni* and *C. coli* need to be quickly spotted in the food chain (EFSA, 2010).

The first known *C. jejuni* epidemic occurred in an Austrian youth camp in September 1998. There were 64 children in all, with 38 showing classic indications of *Campylobacter* gastroenteritis. The bacteria were thought to have come from unpasteurized milk provided by a local dairy. Fecal samples were taken from 20 of the milk-producing cows, and *C. jejuni* was found in five animals, confirming that the outbreak was triggered by consuming unpasteurized milk tainted with *C. jejuni*. The current study aimed to check *Campylobacter* spp. prevalence in milk and various milk products.

MATERIALS AND METHODS

Ethical approval

The Faculty of Veterinary Medicine at New Valley University, Egypt's ethics committees' regulations were followed in terms of animal care and study protocols.

Collection and preparation of samples

A total of 150 random samples were collected of marketable milk (dairy farms and dairy shops, 30 samples each) and some milk products including soft cheeses (Kareish and Domiati) from grocery shops (30 samples each) and ice cream from street vendors and small-scale producers (15 samples each) were gathered from various localities in New Valley Governorate. The samples were collected from March to September 2021. These samples were collected in clean, dry and sterile containers. As quickly as possible, the collected samples were sent to the Microbiology

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laboratory at Faculty of Veterinary Medicine, New Valley University to be evaluated. (APHA, 2004), Regarding cheese samples, they were mixed in a high-speed blender at room temp. not exceeding 24°C. Samples were weighted, tested immediately and mashed thoroughly in a sterile mortar. Finally, ice cream was melted in a container that was placed in a water bath at 40°C for 10 min. to prevent the multiplication of microorganisms.

Isolation of Campylobacter spp. (Salihu et al., 2010)

The appearance of *Campylobacter* spp. was identified in samples using the isolation procedure and selective enrichment described by Roberts and Greenwood (2008). One gram of the homogenized samples was aseptically inoculated into a sterile screw-capped tube including 9 ml of Bolton broth containing Bolton broth selective supplement and 5% laked horse blood, which was incubated under suitable microaerophilic environments in an anaerobic jar by using the Gas Pack System BBL (5% O2 10% CO_2 and 85% N2) at 37°C for about 4 h before increasing the temperature to 41.5°C. After plating a loopful of the incubated broth onto *Campylobacter* blood-free selective agar base and incubating the plates for 48 hours at 41.5°C under proper microaerophilic environments, suspicious colonies were chosen and isolated. Biochemical response tests (catalase test, oxidase test, glucose fermentation test, and hydrogen sulphide formation using iron-containing media) were used to further identify the organism (Cowan and Steel, 1974).

Polymerase chain reaction (PCR)

This part was done at Biotechnology unit of Reference Lab for Veterinary Quality Control on Poultry Production (RLQP), Animal Health Research Institute, Dokki, Giza, Egypt.

DNA extraction

Suspected colonies and standard strains (*C. coli* and *C. jejuni* strains were identified previously by RLQP) were subcultured onto nutrient broth and incubated overnight at 37°C. Qiagen DNA Blood Mini kit (Cat. No. 51104, Hilden, Germany) was used to extract DNA, which was then kept at 20°C until further investigation.

DNA amplification

Preparation of the Emerald Amp GT PCR master mix (Takara) Code No. RR310Akit for PCR, the total volume of the PCR reaction was 25 µl, the reaction was contained the following 12.5 µl Emerald Amp GT PCR master mix (2x premix), 4.5 µl PCR grade water, 1 µl for each forward and reverse primers (20 pmol) and 6 µl template DNA.

The primers cycling conditions during cPCR were started with initial denaturation at 94˚C for 5 min, followed by 35 cycles including the denaturation phase at 94˚C for 30 sec, annealing phase for 40 sec at 58 and 55˚C for *C. coli* and C. jujeni primers, respectively, extension phase at 72˚C for 40 sec. At the end, the final extension was carried at 72˚C for 10 min.

In PCR, oligonucleotide primers are utilized. Metabion (Germany) provided two pairs of primers. The sequence of *C. coli* ceuE primers were AAT TGA AAA TTG CTC CAA CTA TG for forward primer and TGA TTT TAT TAT TTG TAG CAG CG for reverse primer, while the forward primer sequence of *C. jejuni* mapA was CTA TTT TAT TTT TGA GTG CTT GTG and the reverse primer sequence was GCT TTA TTT GCC ATT TGT TTT ATT A (Eunju and Lee, 2009).

Agarose gel electrophoreses (Sambrook *et al*., 1989) with modification

In a sterile flask, electrophoresis grade agarose (1.5 g) was produced in 100 ml TBE buffer, microwaved with agitation to mix all granules, and allowed to cool at 70°C before adding 0.5g/ml ethidium bromide and complete mixing. Warm agarose was put directly into the gel casting device with the required comb in apposition and let to polymerize at ambient temp. After removing the comb, the electrophoresis tank was filled with TBE buffer. The gel was loaded with 20 µl of each PCR amplification sample, negative and positive control. The power supply was between 1 and 5 volts per cm of tank length. After around 30 minutes, the run was halted, and the gel was moved to the UV cabinet. A gel documentation system photographed the gel, and the data was evaluated using a computer software (Alpha Innotech, Germany).

RESULTS AND DISCUSSION

*Campylobacter*s were discovered to be an environmental and health risk in the European Union, causing around 121,000 enteric pathogens in 2020, resulting in significant potential costs (Ramić *et al*., 2022).

Table 1 displays the acquired results, the prevalence of *Campylobacter* spp. in the total tested milk samples were 3.3%. These results were far superior to those obtained by DuzGun *et al*. (2000); EFSA (2006); Kazemeini *et al*. (2011). In addition, these results were extremely lower than those obtained by Roushdy (2000); Yaman and Elmalı (2004); Mahmood *et al*. (2009); Salihu *et al*. (2009); Khanzadi *et al*. (2010); Modi *et al*. (2015) and Zeinhom *et al*. (2021).

The existence of *Campylobacter* spp. in the tested marketable milk samples was ascribed to the milk's insufficient hygienic production and handling environment. Milk contamination during or after milking is most likely of fecal origin, but incorrect cleaning and disinfection treatment of the udder may be the cause. Furthermore, naturally occurring *Campylobacter* mastitis may be a source of *C. jejuni* infection in milk (Ebraheem, 2011).

Various researchers investigated the seasonal *Campylobacter*

Table 1. Prevalence of *C. coli* and *C. jejuni* in marketable milk and some milk products.

Examined sample	No. of the examined sample	Positive of *C. coli*		Positive of *C. jejuni*	
		No.	%	No.	%
Marketable milk	60	1	1.6	1	1.6
Kariesh cheese	30	0	-	1	3.3
Domiati cheese	30	0	-	0	-
Ice cream	30	2	6.6	1	3.3
Total	150	3	2	3	2

spp. prevalence in milk. They discovered that *Campylobacter* spp. dispersion in raw milk during the summer is substantially higher than that during the cold dry season, which had a low rate (Mahmood *et al*., 2009).

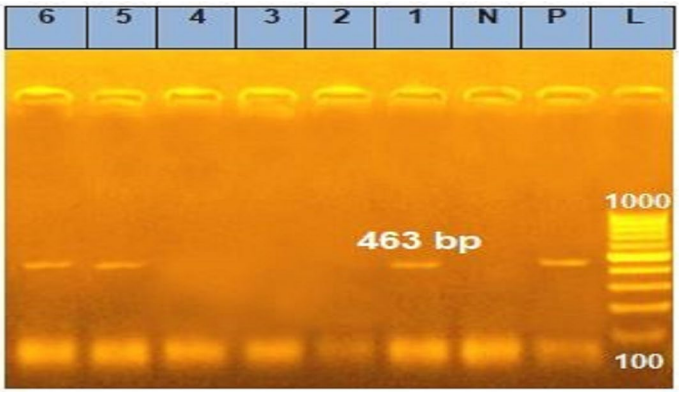

Fig. 1. PCR identification of *C. coli*. Lane (M) DNA ladder 100 bp, lans (1,5,6) positive samples for *C. coli* at 463 bp, lans (2,3,4) negative samples.

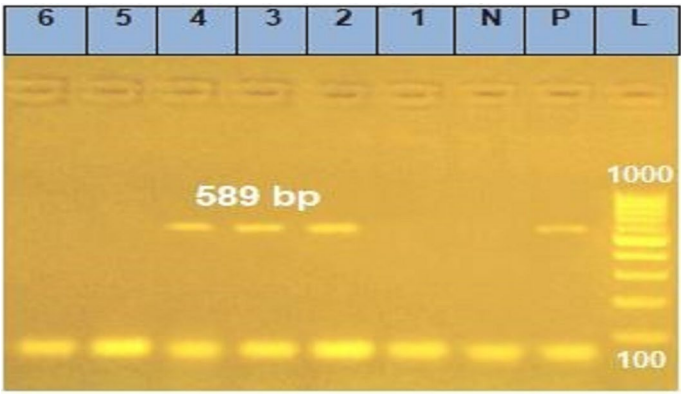

Fig. 2. PCR identification of *C. jejuni*. Lane (M) DNA ladder 100 bp, lans (2,3,4) positive samples for *C. jejuni* at 589 bp, lans (1,5,6) negative samples.

According to the findings in Table 1, the prevalence *Campylobacter* spp. in soft cheese (kariesh cheese) samples was 3.3%. *Campylobacter* spp. not found in Domiati cheese samples. These results were far inferior to those got by El-Shaboury *et al.* (2010); Salem *et al.* (2019) and Zeinhom *et al.* (2021).

Many varieties of soft cheese were implicated in gastroenteritis outbreaks due to *Campylobacter* spp. such kind of cheese was consumed after a short period of maturity, sometimes only a few days. Kariesh cheese is a popular Egyptian food because of its great nutritional value and low cost. It is manufactured at farmers' homes from naturally fermented skim milk, and the fresh product is either consumed by the farmer's family or sold at local markets. Pathogens that enter the product from various environmental sources may contaminate it. The primary source is raw milk (Ahmed, 2004).

One of the most popular frozen milk products in the United States and around the world is ice cream. According to the findings in Table 1, the prevalence of *Campylobacter* spp. in ice cream samples was 10%. A nearly identical outcome was obtained by Saad *et al.* (2007). While these results were extremely higher than those obtained by Mahmood *et al.* (2009) and El-Shaboury *et al.* (2010).

The obtained results showed that the ice cream samples from street vendors were of poor quality. To prevent contamination and protect consumers from infections, high-quality raw ingredients, efficient heat treatment, thorough cleaning and sanitation of equipment, and good hygienic measures throughout the manufacture, handling, storage, transportation, and distribution of ice cream are required.

Different *Campylobacter* species were clearly identified as *C. jejuni* (2%) and *C. coli* (2%). *C. jejuni* isolated from 1(1.6%) marketable milk, 1(3.3%), Kariesh cheese and 1 (3.3%) ice cream. While *C. coli* was detected in 1(1.6%) marketable milk and 2 (6.6%) ice cream (Table 1).

CONCLUSION

According to the findings, marketable milk and milk products (ice cream, Domiati cheese and Kariesh cheese) are vehicles for the transmission of food poisoning bacteria such as *Campylobacter* to humans. As a result, it is advised to consume high-quality raw milk or use it in the manufacture of certain goods. Furthermore, all raw milk used in the making of soft cheese, ice cream, or other dairy products must be pasteurized.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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